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Award Number: DAMD17-00-1-0468

TITLE: Measurement of the Electron Density Distribution of Estrogens - A First Step to Advanced Drug Design

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REPORT DATE: July 2001

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2001	Annual Summary (15 Jul 00 - 14 Jul 01)

4. TITLE AND SUBTITLE
Measurement of the Electron Density Distribution of Estrogens - A First Step to Advanced Drug Design

5. FUNDING NUMBERS
DAMD17-00-1-0468

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

It has been shown that the development of certain types of cancer can be hormone dependent. Estrogens, such as estradiol, have the ability to bind as ligands to the estrogen receptor in the first of many steps which could result in the activation or repression of genes critical in the mechanism of tumor growth. The principle objective of this proposal is to relate known biological reactions to physical properties such as point charges of atoms and the electrostatic potential.

We are obtaining information about these electronic properties of estrogen derivatives from experimental determination of their electron density using high quality single crystal X-ray crystallography. During the past year, significant progress with three of the derivatives (17β-estradiol•½MeOH, 17α estradiol•½H_2O, and 17α-estradiol•urea) have been made. The completion of these, along with the other derivatives being studied within the research group should begin to provide a reasonable pool of data to begin the comparative studies. Continued effort must be made to crystallize more systems, collect data, and further increase the pool of data we have to refer to. This is necessary in order for us to reach our intended goal of developing a new method of advanced drug design.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, x-ray crystallography, estrogens, drug design	7
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Introduction

It has been shown that the development of certain types of cancer can be hormone dependent. Estrogens, such as estradiol, have the ability to bind as ligands to the estrogen receptor in the first of many steps which could result in the activation (agonistic effect) or repression (antagonistic effect) of genes critical in the mechanism of tumor growth. It is the object of this study to relate physical and chemical properties of estrogen derivatives to certain observed biological functions. It is hoped that detailed analysis of X-ray crystallographic data will provide important information to assist in the development of therapeutic drugs. My role is the experimental determination of the electron density distribution of several estrogens as part of a larger study to investigate a wide variety of estrogens.

Body

Task 1. Preliminary studies on a series of crystals of 'A- and D-ring' estrogen derivatives.

- Develop crystallization methods for the derivatives which are not yet available as charge density quality single crystals.
  - I had previously found crystallization methods for the following compounds:
    17β-estradiol • urea
    17α-estradiol • ½ H_2O
    17β-estradiol • ½ MeOH
    17β-estradiol • ⅔ MeOH • ⅓ H_2O

    Continued efforts have been made to explore the seemingly large array of solvent molecules, which can be supported in the crystal systems. Additional work also had to be done on the recrystallization of the 17β-estradiol • urea system. A charge density data set was collected on this system, however the data was not useable. Effort was made to obtain even higher quality crystals and data to alleviate this problem. This proved successful and will be discussed later in the report.

- Temperature studies on each derivative to establish tolerances and the appropriate temperatures for the measurements.
  - Temperature studies were performed on the second batch of 17β-estradiol • urea crystals. They have been consistent with that of the 1st batch of this composition and the other systems listed above. There is no problem with crystal stability when approaching liquid nitrogen temperatures. Continued work has also been done to try to find a better method of cooling crystals down to near liquid He temperatures.

- Preliminary routine X-ray crystal structure determination on each derivative to check for composition, quality, and solvation.
  - The structure of the 17β-estradiol • ⅔ MeOH • ⅓ H_2O system, while chemically interesting, is not a good candidate for charge density research. The extreme

number of atoms in the unit cell makes a detailed charge density study of the system very difficult. This system is, in fact very important chemically, because the three independent molecules in the asymmetric unit are not identical. While they share the same stereochemical configuration, they differ slightly in physical geometry. This may be important in helping to relate the molecule to the activity observed. A manuscript is currently being prepared discussing the geometry of these molecules, and its possible link to activity (Appendix A).

- All other systems listed above have already moved beyond this point in the study.

Task 2. Electron density quality data collection on the above mentioned estrogen analogues.

- X-ray diffraction studies at liquid nitrogen temperatures on crystals that did not qualify for lower temperatures.
  - While it was reported last year that a charge density study of 17β-estradiol • ½ MeOH had already been collected, given the system is P1 with 2 molecules in the unit cell it has proven a very difficult system to work with. This will be further discussed later.
  - A complete data set of 17α-estradiol • ½ H_2O has been successfully collected and is currently being analyzed.
  - A complete data set of 17β-estradiol • urea had also been collected. As stated earlier this data was not useable. Due to the large unit cell parameters and mosaicity of the system, reflection overlap required recollection of the data. A second data set has been collected from a different batch of crystals. The quality of the data has yet to be determined. The data was collected on a new detector system from Bruker Analytical X-ray Systems and we have been working closely with them to determine the quality of their detector related to charge density studies of small molecules.

- X-ray diffraction studies at liquid helium temperatures.
  - Work has been continued to try to find a better strategy for cooling crystals down to near liquid He temperatures.

Task 3. Interpretation and analysis of nitrogen and helium temperature charge density data sets of above mentioned estrogen analogues.

- Analysis of the experimental data.
  - 17β-estradiol • ½ MeOH: Data treatment has been performed, which includes integration, absorption correction, data scaling, and statistical outlier determination. In order to perform a multipole refinement, a proper coordinate system must be set up to include necessary dummy atoms and to utilize the most appropriate multipoles. This has been done for this molecule. Initial spherical atom refinements to obtain the best positional and thermal parameters have also been finished. Initial refinements including population of multipole parameters have been started. The fact the crystal system is P1 means that there is no symmetry equivalent data which reduces redundancy in the data. This increases the difficulty and requires more careful treatment and interpretation of the data. Despite this, the outcome looks very promising.

- 17α-estradiol • ½ H_2O: This data has already been treated, coordinate system has been set up, and taken to the stage of multipole refinement. Initial results indicate that this refinement will conclude successfully.
- 17β-estradiol • urea: As stated earlier, the quality of the second data set is as yet undetermined. Several methods of data reduction have been performed. Because of uncertainties associated with the new detector, several of these trials have been taken to the point where evaluation based on the multipole refinement can begin. Multipole refinements on a few of these trials have already been started.

- Comparison of the results from the series of estrogen analogues.
  - Once the evaluation of these three systems is complete, the results can be compared to each other as well as the systems studied by the other members of the research group.

- Analyze relationship of charge density to receptor binding affinity and the chemical/biological effects as related to breast cancer.
  - This step can not be started until a sufficient amount of charge density studies have been completed successfully.

- Final analysis and preparation of manuscripts.
  - Manuscripts will be completed as work progresses.

Key Research Accomplishments

- Crystallization of high quality crystals
- Progress in the study of 17β-estradiol • ½ MeOH
- Acquisition of quality data and progress in the study of 17α-estradiol • ½ H_2O
- Acquisition of quality data and progress in the study of 17α-estradiol • urea
- Continued development in cryogenic techniques, software and hardware development, as well as methodological development in charge density technique and evaluation.

Reportable Outcomes

- Annual American Crystallographic Association Meeting (July 2000)
- Annual European Crystallographic Meeting (August 2000)
- Gordon Research Conference – Electron Distribution and Chemical Bonding (July 2001)
- Manuscript being prepared discussing 17β-estradiol • ⅔ MeOH • ⅓ H_2O system

Conclusion

The previous work of this group, including myself, has shown it is possible for molecules of this size to be studied using charge density analysis. During the past year, I have made significant progress with three of the derivatives (17β-estradiol • ½ MeOH, 17α estradiol • ½ H_2O, and 17α-estradiol • urea). The completion of these, along with the other derivatives being studied within the research group should begin to provide a reasonable pool of data to begin the comparative studies. Continued effort must be made to crystallize more systems, collect data, and further increase the pool of data we have to refer to. This is necessary in order for us to reach our intended goal of developing a new method of advanced drug design.